Synthetic Modification of Seed-Derived Oils Utilizing Reaction Chemistry in Supercritical Fluids

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Introduction

Environmentally acceptable manufacturing processes, including chemical synthesis, have been the recent focus of a wave of new technology commonly called "green chemistry" (1). Among the technologies addressing this problem are supercritical fluid technology and its many variants: supercritical fluid extraction (SFE), supercritical fluid fractionation (SFF), supercritical fluid chromatography (SFC), and supercritical fluid reactions (SFR). Actually, the supercritical fluid state has been used previously for assorted synthetic purposes, such as polymerization, enzymatic catalysis, conversions in supercritical water (SC-H_2O), heterogeneous catalysis, pyrolytic and photolytic reactions, and for specific cases in analytical chemistry (2,3).

In addition to the obvious advantage of conducting reactions in environmentally benign supercritical carbon dioxide (SC-CO_2) (4), the supercritical fluid state offers some unique advantages that are inherent in the physicochemical properties of the processing media and are the effect of pressure on reaction rate constants (5). For example, reactant and product mass-transfer rates are substantially improved in supercritical fluids, resulting in transport properties (i.e., diffusion coefficients) and numbers that are more attractive compared to those found in the liquid state. Alteration of fluid density also allows subtle control of reactant or product solubility in the dense transport media as well as control of the product distribution. In some specific cases, it is also possible to conduct reactions at low temperatures in a non-oxidative environment (CO_2), thus protecting compounds that would be altered in more severe thermal environments. By integration of the use of supercritical fluids in the extraction and/or fractionation mode with SFR, the scientist or engineer has considerable flexibility in designing a totally integrating "green" process. For example, catalyst regeneration can in some cases be affected by using the reaction medium after SFR (6).

The purpose of this paper is to illustrate how supercritical fluid processing can be applied to great advantage in the conversion of seed-derived oils to useful industrial chemicals. Here the authors report on the results of a 2-year research program in which reaction chemistry in SC-CO_2 has been integrated with both SFE and SFF. A major focus has been enzyme utilization to convert oils into useful derivatives, reactions that can also be used for analytical purposes. In certain instances high-pressure CO_2 can act as a catalytic agent, thereby avoiding the use of heterogeneous catalysts that must be removed from the product mixture after reaction completion. Enrichment of specific components in a product mixture can also be affected by SFF, and the authors have applied a thermal gradient column to enrich the monoglyceride content of a synthetic glyceride mixture. Finally, some preliminary results on fatty acid hydrolysis in subcritical water seem to offer a better understanding of classic hydrolysis procedures that have been the synthetic mainstay of fatty acid production for over seven decades (7).

The enzyme-catalyzed reactions reported in this chapter deserve further comment since they illustrate the versatility of a single enzyme in offering multiple synthetic possibilities. One enzyme performed transesterifications, glycerolysis, interesterifications, and could be optimized to hydrolyze oils to fatty acids if required. The inherent reproducibility of the lipolysis reaction to produce methyl esters has found a dual niche in this research, permitting generation of biodiesel (8) and derivatives that find application in fat analysis according to the regulations promulgated by the Nutritional Labeling and Education Act (NLEA) (9).

Materials and Methods

Considerable experimental detail can be found in the publications on which this overview of the synthetic program is based. This section will present only some of the general materials and methods that have been employed in these studies, as well as more specific details on certain operations that have not yet appeared in print. This will include a discussion of the Novozyme 435 preparation that was found so useful in these studies, generic descriptions of enzymatic reactor assemblies utilized to conduct the reactions, a description of conditions specific for the enzymatically induced glycerolysis and intraesterification reactions, the thermal gradient fractionation column, and the batch reactor used for hydrolysis in subcritical water.

Properties and Use of Novo Nordisk Novozym 435

The Novozyme 435 immobilized enzyme was obtained from Novo Nordisk (Danbury, CT). It is capable of acting as a triacylglycerol hydrolyze as well as a carboxylesterase; both reaction modes can be conducted in the presence of SC-CO_2.

[1]Names are necessary to report factually on data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may be suitable.

The lipase demonstrates both nonpositional and 1,3-specificity, but the authors employed the former more than the latter mode in these studies. It is a genetically engineered enzyme, the recombinant gene being derived from *Candida antarctica* and transferred into *Aspergillus oryzae*. Immobilization is affected on an acrylic resin, 0.3–0.9 mm particle diameter, with a bulk density of 430 kg/m^3. The resin can be easily poured into the extraction and reaction vessels, in which it is held in place by glass wool plugs.

Enzyme activity is measured in terms of synthesized propyl laurate units per gram and is rated at 7000 units. To test the efficacy of Novozym 435 in performing this conversion, the reaction between lauric acid and *n*-propyl alcohol was performed at 34.5 MPa and 50°C in SC-CO_2; it achieved a 93.4% conversion in 15 min. This indicates that enzymatic activity is maintained in the presence of SC-CO_2 and that an esterification reaction can be conducted quite rapidly under these conditions.

Another factor affecting enzymatically catalyzed reactions in supercritical fluids is the moisture content of the system and that associated with the enzyme. The water content for commercial Novozym 435 preparations is 1–2 wt%. Karl Fischer titrations indicated that the Novozym 435 received contained approximately 1 wt% water. For the synthesis reported in this research, it was found that neither water level in the reactor, nor that adsorbed on the Novozym 435 appreciably decreased the enzyme's activity under supercritical conditions. The enzyme preparation retained its activity over many reuse cycles and on numerous substrates with variable moisture content. This latter observation may be due to the small, but finite solubility of water in SC-CO_2 at the extraction/reaction pressure and temperature quoted previously (10). It was found that the Novozym 435 preparation could be used at temperatures up to 60°C with no loss of enzyme activity.

Enzymatic Reactor Configuration and Reaction Conditions

Two scales of extractor/reactor were utilized in the enzymatic catalyzed studies. The first employed a modified Isco SFX-2-10 SFE unit (Isco, Inc., Lincoln, NE) that utilizes extraction cartridges, of approximately 10 mL in volume. Carbon dioxide was supplied by a dual syringe pump arrangement, while separate syringe pumps delivered the vegetable oil and alcoholic reagent (Fig. 1). The oil dissolved in the SC-CO_2/alcohol mixture was then passed over the enzyme bed where it was converted to the transesterified product. Additional details on this device are described in the literature (11). Extraction of food samples with subsequent conversion of lipid extracts to methyl esters utilized a similar approach, except that the food sample was placed in the extraction cell before the enzyme bed. For analytical purposes, it was found necessary to remove moisture in high water content foods by freeze drying, prior to conducting the previously described derivatizations.

Scaling up the transesterification scheme was also enacted in order to obtain more synthetic product for physical and chemical characterization. This approach utilized larger extraction and reaction vessels made from high-pressure tubing (Autoclave Engineers, Inc., Erie, PA) and had dimensions that were reported previously (11). In this apparatus, the enzyme was also contained in a separate tubular reactor downstream from the extractor vessel, while alcoholic reagents were added with the aid of a high-performance liquid chromatography (HPLC) pump (Altex Model 100, Beckman Instruments Co., Fullerton, CA). Carbon dioxide was delivered via a gas booster pump (Haskel Co., Burbank, CA).

A similar device was also used to perform the glycerolysis reactions in flowing SC-CO_2 over Novozym 435. Here the appropriate oil to glycerol ratios were pumped into the flowing CO_2 stream by Isco 100 syringe pumps and subsequently transported over 10 g Novozym 435 contained in a 0.8 cm. i.d. × 10.2 cm vessel. The resulting mixed glyceride product then exited through a micrometering valve into a round-bottomed flask. The entire assembly was placed in a gas chromatographic oven to avoid product precipitation before reaching a receiver flask. Similarly, interesterification reactions were performed using separate tubular extractor and reactor vessels in the same chromatographic oven. In this case, approximately 16 g oil was mixed with 12 g Celite 521 and poured into a 1.7 cm i.d. × 23 cm extraction vessel. This was placed ahead of a second vessel having the same dimensions containing between 1 and 20 g of Novozym 435. Extractions/reactions were performed at 27.5 MPa and 70°C. Using this apparatus permitted the synthesis of multigram quantities of products necessary for physical and chemical characterization.

Thermal Gradient Fractionation Column

The alternative glycerolysis reaction performed in an autoclave under SC-CO_2 has been previously described (12). Glyceride mixtures from both the enzymatic as well as the batch autoclave experiments could be further fractionated with a thermal gradient fractionation column. The pilot-scale column used in these studies consisted of a preheated section followed by four separately heated zones, each with an internal diameter of 1.43 cm and a height of 41.5 cm. The total column volume was 260 cm^3; each section was packed with 0.16 ProPak distillation packing (Scientific Development Company, State College, PA), providing a 94% void volume in the column proper. Each zone was heated by Glas Col heating mantles (Glas Col, Inc., Terre Haute, IN), and temperatures were monitored by Type J thermocouples. The glyceride-feed mixture was Myvatex Mighty Soft Softener obtained from Eastman Chemical Co. (Kingsport, TN); it was delivered to the top of the first heated section of the column with the aid of a Haskel Model HS-188 liquid pump operating under stroke counter control.

The reaction vessels operated semicontinuously. For each batch, approximately 70 mL feed was delivered to the column before fractionation commenced, following an initial 1-h equilibration period. Carbon dioxide was fed to the column via a Haskel Model ACT 62/152 booster pump; the fractionation was conducted over a 2-h time period. Top product was collected after expansion through a micrometering valve located at the top of the column at an expanded fluid flow rate of 2 L/min. The optimal temperature gradient was found to be 65–95°C, with approximately 10°C between the four heated zones comprising the fractionating column.

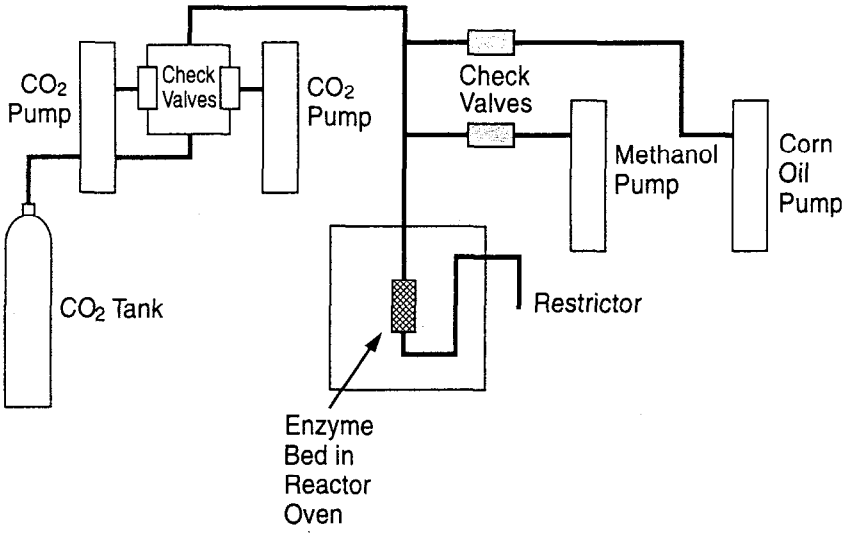

Fig. 1. Schematic of the continuous flow system for the synthesis of fatty acid methyl esters from seed oils.

Hydrolysis Experiments in Sub- and Supercritical Water

Vegetable oil hydrolysis in sub- and supercritical water media was accomplished in a batch reactor consisting of a 316 SS tube (20.3 cm × 1.43 cm i.d.). One end of the reactor was capped, while the other had a Type J thermocouple encased in an Inconel sheath to measure the internal temperature of the reactor. The entire vessel had a volume of 35.5 mL and was contained in a converted gas chromatographic oven for temperature control. After the reaction was conducted, the vessel was removed from the GC oven and cooled by two opposing air knives (Exair-Knive #2012, Exair Corporation, Cincinnati, OH). Typical reaction conditions consisted of charging the vessel with 25 mL water and 4 mL vegetable oil before it was placed in the oven and heated to the desired temperature. Cool-down times were approximately 10–13 min to reach 35°C, with subsequent product workup achieved by oleophilic acid partition between the aqueous layer (Na_2SO_4 added) and diethyl ether.

Results and Discussion

Utility of Transesterification Reactions

Transesterification of vegetable oils in flowing SC-CO_2/methanol over Novozym 435 yields quantitative conversion of the oil to methyl esters and glycerol. The glycerol produced is also solvated by the SC-CO_2/methanol mixture and can be collected as a separate phase from the methyl esters. Precision and accuracy of the fatty acid methyl ester (FAME) distribution from the lipase-catalyzed transesterification was comparable to a FAME distribution achieved by BF_3/methanol reagent (11). This suggested the use of this approach for analytical FAME analysis. Table 1 shows the results for soybean oil (SBO) esterification performed in triplicate. The average of these results is comparable to the result achieved for biodiesel produced by a sodium-catalyzed reaction.

TABLE 1 Fatty Acid Methyl Esters from Soybean Oil Using Lipase Catalysis Under Supercritical Fluid Conditions

Fatty Acid	Run #1	Run #2	Run #3	Avg.	RSD[a]	Soy Diesel
Palmitic	12.1	11.3	11.5	11.6	2.83	10.6
Stearic	4.8	5.2	5.2	5.1	3.14	4.5
Oleic	23.9	24.3	23.8	24.0	0.91	23.7
C18:1d11	1.53	1.54	1.51	1.53	0.72	1.29
Linoleic	52.0	52.3	52.3	52.2	0.33	51.1
Linolenic	5.6	5.4	5.6	5.6	2.02	7.1

[a]Relative Standard Deviation.

The high precision and accuracy achieved by lipase-catalyzed transesterification of seed oils allowed application of the reaction to analyze the fat content of foods. The esterification reaction can be run on the Isco SFX-2-10 system mentioned previously, or alternatively on a highly automated SFE system connected in tandem to a gas chromatograph (GC) (13). Using the latter system, the total, saturated, and monounsaturated fatty acid content of meats have been determined with the aid of a robotic transfer arm, operating between a Hewlett Packard Model 7680 supercritical fluid extractor receiver tray and the injection vial tray of the GC unit. Results for the determination of total fat content on several different meat samples having different fat levels are presented in Table 2. These results are in reasonably good agreement with those determined by standard solvent-extraction methods used in a new NLEA protocol (14). Recent results have indicated that Novozym 435 is capable of interesterifying other lipid moieties, such as phospholipids and cholesteryl esters, yielding extraction recoveries better than 95% and even higher reaction conversion efficiencies.

Glycerolysis over Novozym 435

Initial screening studies conducted on the Isco SFX-2-10 extractor indicated that corn, cottonseed, olive, and soybean

TABLE 2 Comparison of Total Fat Results from SFE/SFR Method and Conventional Solvent Extraction Method

Sample	Total Fat - SFE/SFR		Total Fat Solvent Extraction	
	Wt%	RSD[a]	Wt%	RSD[a]
Bacon	39.4	3.4	38.7	1.7
Beef (low fat)	11.2	5.5	12.8	5.6
Beef (medium fat)	20.6	2.3	21.8	4.5
Beef (high fat)	28.8	1.2	28.6	3.7
Ham (low)	9.9	5.5	10.0	3.0
Ham (high)	16.5	4.1	17.1	0.8
Sausage (low)	11.1	6.8	9.1	2.3
Sausage (medium)	15.8	3.7	13.9	3.3
Sausage (high)	20.6	6.1	20.3	2.2

[a]Relative Standard Deviation, $n = 3$.

oils underwent glycerolysis readily at pressures of 20.7–34.5 MPa and temperatures of 40–70°C. These conditions were optimized for soybean oil at 27.6 MPa and 70°C. When utilizing a lipase during a glycerolysis reaction, it is important that the water level in the reactor be controlled and the moisture level in the reactants be assessed. Karl Fischer titration on the Novozym 435 preparation revealed a 1.4 wt% moisture level, while the moisture level on different glycerol lots employed in the glycerolysis experiments ranged between 0.7 and 4.2 wt%.

The disadvantage of high water levels in the reactor are twofold. Excessive moisture inhibits enzyme activity (i.e., the glycerol containing 4.2 wt% reduced the enzyme's activity to only 60% of the activity exhibited in the presence of 0.7 wt% water) as shown in this study and by others (15). In addition, the presence of water promotes the competitive reaction of hydrolysis, thereby facilitating the formation of fatty acids. A kinetic time lag in the reaction may also be exhibited until the enzyme's water content is partially removed by the flowing SC-CO_2 (16).

The soybean oil reaction with various alcohols was also compared in this study and found to parallel the solubility of the alcohols in SC-CO_2. Curiously, it was found that the reaction with alcohols, such as 1,2-propanediol, and glycerol occurred despite alcohol and soybean oil feed rates that exceeded their solubilities in CO_2. This suggests that a multiphase system is present in the fluid phase that does not inhibit glycerolysis.

Similar results were also obtained in reacting soybean oil with ethylene glycol, but the presence of ethylene glycol also tended to denature the enzyme. The relative activity of Novozym 435 towards alcoholic reactants was 100:90.6:53.4:2.2 for methanol:1,2 propanediol:ethylene glycol:glycerol, respectively.

The effect of the glycerol water content on the glycerolysis product distribution is shown in Table 3. Obviously the lower water content results in more monoglycerides being produced and offers product distribution control. This variable, along with the ability to adjust the flow rates of reactants into the SC-CO_2 stream, offers considerable versatility in producing useful glyceride mixtures for industrial use and is the subject of a recent patent application (17).

TABLE 3 Effect of Glycerol-Water Content on Product Distribution at 70°C and 27.6 MPa

Product	0.7% Water	4.2% Water
Fatty Acids	—	1
Monoglycerides	84.0	67.0
Diglycerides	15.4	28.9
Triglycerides	0.6	3.1

Randomization of Fats/Oils in Flowing SC-CO_2

As noted in the Materials and Methods section, intraesterification of seed oils can be conveniently conducted in flowing supercritical fluid streams, however, the production rate is limited by oil solubility in SC-CO_2 at a given pressure and temperature. Larger enzyme beds will also be required as the oil solubility increases with temperature and pressure (18); this will also require both a thermophilic and pressure-resistant enzyme preparation. Nonetheless, the production concept is valuable, particularly in generating oil mixtures with tailored physical properties targeted for use in the food industry.

The results can be quite striking between the oil entering the lipase high-pressure reactor and that exiting after randomization has been accomplished. For a palm olein composition, the change is from a partially liquid oil at room temperature to a semisolid under the same conditions after intraesterification. Table 4 shows dropping point data before and after intraesterification in the SC-CO_2 /lipase reactor. For all of the oleophilic compositions listed, except for tallow and the tristearin/soybean oil mixture, the dropping point is greater than for the initial starting oil/fat. Similarly, measurement of the solid fat content (SFC) by wide line NMR on native and intraesterified palm olein and a genetically engineered high-stearate soybean oil, showed that the randomized oil had a higher SFC than the starting oil as the temperature increased. This data indicates that such synthesized mixtures may have utility in margarine formulations.

A chemical rationalization of this effect can be provided by analyzing the mixed glyceride compositions, before and after randomizations, with the aid of HPLC. As shown in

TABLE 4 Comparison of Dropping Points of Various Fats and Oils Before and After Randomization in Flowing Supercritical Carbon Dioxide

Sample	Initial Dropping Point (°C)	Final Dropping Point (°C)
RBD Soybean oil[a]	0.9	10.7
HS-1 Soybean oil[b]	18.3	27.6
A-6,3 Soybean oil	20.5	30.4
Palm olein	21.7	37.7
Cocoa butter	32.0	41.6
Tallow	41.3	38.8
20% Tristearin/80% soybean oil	60.4	37.1

[a]Refined, bleached, deodorized soybean oil.
[b]High-stearate soybean oil.

Fig. 2, there is a change in relative composition of the glyceride components for palm olein, particularly in the increase of the LOP, SOO, and POO triglycerides in randomized palm olein, relative to the levels found in palm olein. Figure 3 compares the HPLC profiles for the high-stearate soybean oil with the randomized product after passage through the lipase reactor bed. There are subtle changes in the triglyceride composition that have a considerable impact on the physical properties of the oil before and after intraesterification. Both of the previously mentioned oils show losses in the disaturate glyceride along with a concomitant increase in tri- and diunsaturate triglycerides. The loss of disaturate functionality has been shown to aid in fat crystallization (19).

Glycerolysis in SC-CO_2 Medium

The authors have recently published the details and results of conducting vegetable oil glycerolysis in the presence of SC-CO_2 (12). However, it is worth noting the benefits occurring from utilizing this method which is partially based on

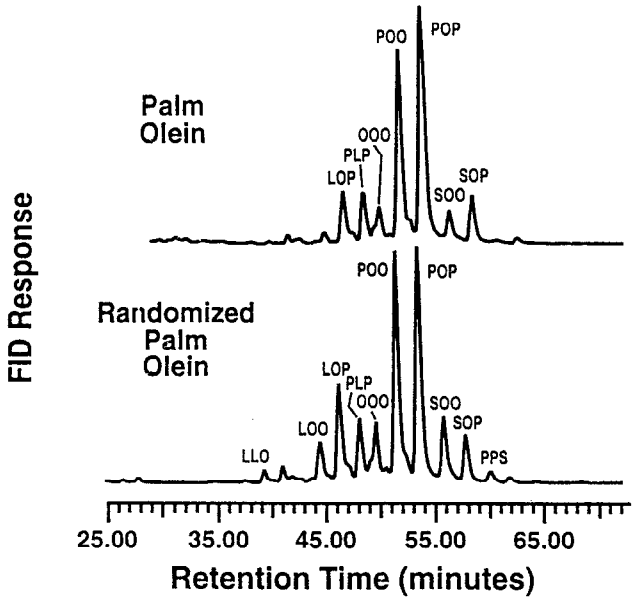

Fig. 2. RP-HPLC of palm olein fraction before and after randomization.

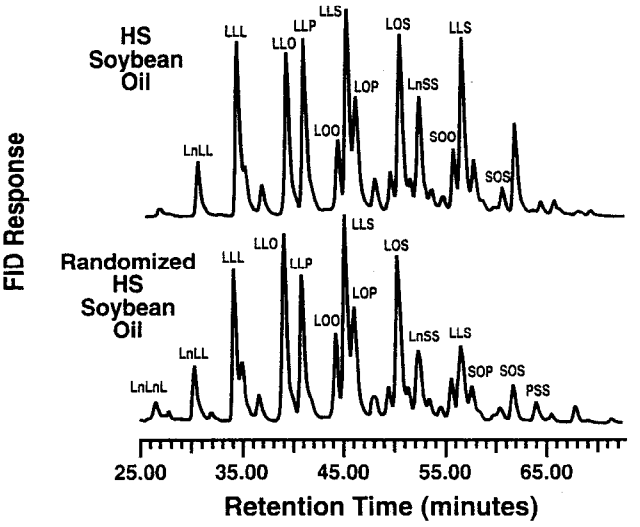

Fig. 3. RP-HPLC of high-stearate soybean oil before and after randomization.

preliminary research conducted by Indian scientists (20). Like these researchers, the authors found that glycerolysis may be conducted advantageously by employing pressurized carbon dioxide, thus avoiding the use of solid catalysts and the attendant problems of their disposal after the conclusion of the reaction. It was also found that the resultant mixed glyceride product (containing approximately 35–45 wt% monoglyceride) is white in color, in stark contrast to darker mixtures produced by conventional chemical processes.

These studies have examined the effect of CO_2 pressure in the supercritical fluid state (at 250°C) and shown that there is an optimal pressure, 20.7 MPa, that favors monoglyceride production (Fig. 4). These yields are higher than those previously attained at near ambient pressures (20) and at higher pressures of SC-CO_2. Apparently, carbon dioxide has a synergistic effect on monoglyceride production and increases the production rate at 20.7 MPa. However, reaction times are typical for those encountered in stirred reactor glycerolysis (Fig. 5), taking almost 4 h for equilibrium concentrations to be reached. It should also be noted that the presence of a small quantity of water in the glycerol used produced free fatty acids from the competitive hydrolysis reaction (Fig. 5).

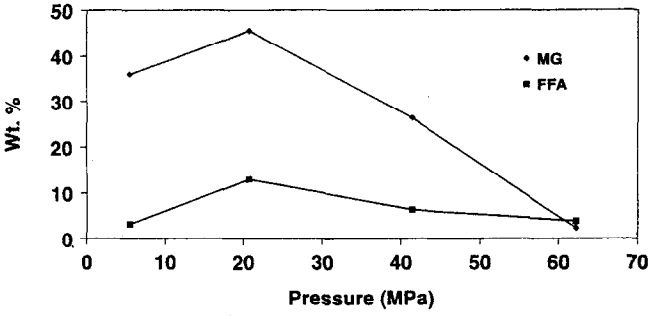

Fig. 4. Effect of pressure on monoglyceride (MG) formation and free fatty acids (FFA). Conditions: 250°C, glycerol/water = 25, and 4% water addition.

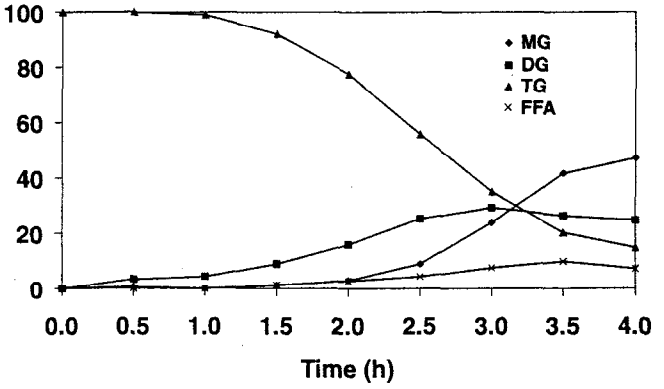

Fig. 5. Composition of the oil phase during glycerolysis as a function of time. MG = monoglycerides, DG = diglycerides, TG = triglycerides, FFA = free fatty acids. Conditions: 250°C, 20.7 MPa, glycerol/water = 25, and 2% water addition.

Table 5 shows the synthesis of monoglyceride-containing mixtures from several commodity vegetable oils employing the reaction conditions mentioned previously. The monoglyceride component ranges from 49.2 wt% for glycerides derived from soybean oil to 41.1 wt% in the case of cottonseed oil. These results were attained at a reaction temperature of 250°C, a reactor pressure of 20.7 MPa, using a glycerol to oil ratio of 25, and the addition of 4% water to the stirred autoclave. Such results indicate that SC-CO_2 may also have an autocatalytic effect on the glycerolysis conducted in the presence of a lipase discussed previously.

Fractionation of Glyceride Mixtures

Monoglyceride enrichment in synthetic product mixtures obtained from the previously discussed processes provides another supercritical fluid processing alternative. As shown in Fig. 6, packed tower fractionation does not necessarily require prohibitively high processing pressures to obtain significant monoglyceride enrichment in these feed mixtures. Enrichment of the monoacylglyceride (MAG) component relative to the diacylglyceride (DAG) and triacylglyceride (TAG) components can be achieved at pressures as low as 17.5 MPa. One may also choose higher pressures to enrich a mixture in DAG or TAG components. Enrichment of MAG from a 40 wt% level to over 90 wt% level can be achieved by this process. The top product is also white in color, similar to that obtained for lower MAG-containing mixtures in the stirred autoclave reactor experiments. This indicates that the white-colored products in both cases can be attributed to the presence of SC-CO_2 in the processing scheme. The color of the top product obtained in the fractionating tower experiments is also equivalent to that observed in 90%+ MAG commercial products.

Top product yields from the fractionating tower are approximately linear with respect to pressure. This indicates that there is a trade-off between separation efficiency and product yield as is often the case in separation technology (21). Interestingly, significant MAG enrichment can be achieved with an isothermal column temperature (90°C), indicating the role that solute vapor pressure plays in achieving the reported fractionation. However, maximum top product yields were achieved at an isothermal column temperature of 65°C, indicating the importance of CO_2 density on the fractionation process. The achieved MAG, DAG, and TAG separation factors appear to be independent of CO_2 flow rates from 2–12 L/min (decompressed), while top product yield/kg CO_2 was found to decrease as the expanded CO_2 flow rate increased.

Hydrolysis of Vegetable Oils in Subcritical H_2O

Experiments on vegetable oil hydrolysis using high-temperature, pressurized water were initially conducted to gain insight into optimizing an acceptable hydrolysis temperature consistent with avoiding substrate oil degradation. Table 6 displays the reaction times and temperatures required to achieve over 97% conversion of the corresponding oil triglycerides to fatty acids in these batch experiments. In general, these conversions took 15–20 min at temperatures of 270–280°C. At these conditions, water density is approximately 0.7 g/mL. As shown for linseed oil, a 20°C drop in temperature results in a lengthening of the reaction time 3.5-fold.

Gas chromatographic fatty acid methyl ester analysis was performed on a number of the resultant fatty acid mixtures to determine if any chemical decomposition or conversion had occurred on these moieties at these temperatures. Both saturated and unsaturated fatty acids remained relatively unaffected below 300°C, although linolenic acid was slightly affected and converted from its original *cis,cis,cis* isomer form. Extreme temperatures, such as 375°C, close to the critical temperature of water (T_c = 374°C), produced a dark brown mixture. Oil analysis by GC-FAME revealed the presence of severe decomposition and/or polymerization of the constituent fatty acids. Currently, studies are being conducted on oil hydrolysis using a flow reactor.

Acknowledgments

The authors gratefully acknowledge the assistance and skill of Jeff Teel in assembling many of the experimental apparatus used in these studies. Thanks are also due Janet Snyder, Fred Eller, and William Neff for certain analyses quoted in this paper.

TABLE 5 Weight Percent Composition of Glycerolysis Products Obtained from Different Vegetable Oils During Glycerolysis in the Presence of SC-CO_2[a]

Oil Type	Monoglycerides	Diglycerides	Triglycerides	Fatty Acids
Soybean	49.2	26.6	10.1	14.0
Peanut	46.6	32.1	12.5	8.8
Cottonseed	41.1	35.0	12.6	11.3
Corn	45.6	32.3	13.0	9.2
Canola	41.7	33.0	16.0	9.3

[a]Conditions: 250°C, 20.7 MPa, glycerol/oil ratio = 25, 4% water addition, and time = 4 h.

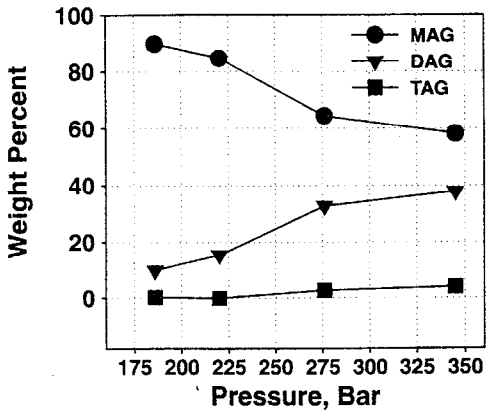

Fig. 6. Effect of pressure on thermal gradient column glyceride fractionation. Conditions: Flow = 2 L/min, Gradient = 65–95°C.

TABLE 6 Triglyceride Conversion to Fatty Acids Dependence on Reaction Time and Temperature

Oil Type	Reaction Time (min)	Reaction Temperature (°C)
RBD Soybean[a]	20	270
Hydrogenated soybean	15	280
Linseed	20	280
Linseed	69	260
Coconut	15	270

[a]Refined, bleached, deodorized soybean oil.

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